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(54) Title: EXTRACTION OF FRESH LIQUID EGG YOLK

(57) Abstract

This invention provides a novel process for the separation of fresh liquid egg yolk into a yolk protein fraction, neutral egg oil fraction and an egg lecithin fraction by treating fresh liquid egg yolk with aqueous ethanol at an elevated temperature to provide a slurry, filtering the slurry to provide solid yolk protein and an aqueous ethanolic filtrate, and thereafter subjecting this filtrate to low temperature crystallization to provide a crystalline neutral egg oil fraction and removing the crystalline fraction to provide a residual filtrate which is an aqueous ethanolic solution containing egg lecithin.

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EXTRACTION OF FRESH LIQUID EGG YOLK

Technical Field

This invention relates to the extraction of fresh liquid egg yolk and particularly the isolation of lecithin from fresh liquid egg yolk of hens eggs and more especially it relates to the isolation of lecithin (phospholipids), neutral egg yolk oil (neutral lipids which are mainly triglycerides) and egg yolk protein from fresh liquid egg yolk of hens eggs.

Industrial Applicability

Lecithin is useful in the food and pharmaceutical industry while egg oil is useful in the preparation of infant formula food because of its fatty acid composition. Egg yolk protein is useful as a source of protein.

Background Art

U.S. patent No. 4,465,693 issued August 14, 1984 describes the purification of crude lecithin from soybean or egg by treating a solution of the crude lecithin in ethanol with 50% or more of water to remove undesired impurities.

U.S. patent No. 4,452,743 issued June 5, 1984 describes the separation of oils and phosphatidylethanolamine from phosphatidylcholine products by means of a chromatographic purification process on a silicic acid gel column at a temperature less than 60°C wherein the starting material is dissolved in an aqueous alkanol such as aqueous ethanol.

U.S. patent No 4,425,276 issued January 10, 1984 describes the preparation of highly purified phosphatidyl-choline free from oils and phosphatidylethanolamine by placing the starting material as a solution in an aqueous alkanol at the head of a chromatographic column of silicic acid gel at a temperature of 40°C to 90°C and eluting the column with an aqueous alkanol.

U.S. patent No. 4,443,378 issued April 17, 1984 describes the use of 95% ethanol in the separation of acylated phospholipids in a chromatographic purification procedure.

U.S. patent No. 4,496,486 issued January 29, 1985 describes the purification of crude phosphatides, e.g. derived from soybeans, peanuts, sunflowers or rape, by means of an aqueous alcohol technique to produce phosphatidylcholine having a low oil content.

British patent specification No. 1,113,241 published May 8, 1986 describes the treatment of a crude undefatted vegetable phosphatide e.g. from soybean oil or rapeseed oil, by extraction with an 80% to 95% aqueous alkanol in an amount of from 2 to 10 litres per kilogram of crude phosphatide at a temperature of from 10°C to 30°C to produce a product containing phosphatidylcholine and phosphatidylethanolamine in a specified ratio range.

Canadian patent No. 1,064,907 issued October 23, 1979 describes the treatment of dried egg yolk powder with aqueous 95% ethanol at a temperature of 25° to 30°C for 2 to 4 hours to remove at least 70% by weight of cholesterol contained in the dried egg yolk powder.

Brief description of the drawing

The drawing is a schematic flow sheet of the process showing the separation of fresh liquid egg yolk into solid yolk residue (protein), neutral egg oil (triflycerides) and egg lecithin (phospholipids).

Disclosure of invention

We have now found, and herein lies our invention, that fresh liquid egg yolk from hens eggs can be subjected to a unique novel process using a precise combination of solvent and temperature to provide a high yield of egg yolk protein, lecithin (phospholipids) and neutral egg yolk oil (triglycerides).

Thus according to our invention we provide a

process for the separation of fresh liquid egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating fresh liquid egg yolk with aqueous ethanol at an elevated temperature to provide a slurry thereof, separating the slurry into a solid fraction and a liquid fraction, for example by filtration, to provide a solid fraction which is solid yolk protein and a liquid fraction which is an aqueous ethanolic solution, and thereafter subjecting the aqueous ethanolic solution to low temperature crystallation to provide a crystalline neutral egg oil fraction and removing this crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

Fresh liquid egg yolk from hens eggs contains about 50% water and about 50% solid matter and the latter is essentially made up of yolk protein and lipids. These lipids are a complex mixture of triglycerides (also called neutral lipids or neutral egg oil) and phospholipids which are polar lipids containing lecithin, together with cholesterol. It is possible to use the process of the present invention to isolate first the yolk protein essentially free from lipids and thereafter to separate the residual lipids into a neutral lipid fraction known as neutral egg oil (triglycerides) and egg lecithin which is essentially phospholipids, i.e. polar lipids containing lecithin. These products thus obtained by the process of this invention are essentially free from, or contain only very minor amounts of, cholesterol.

The drawing shows a preferred embodiment of the process wherein 1 part by weight of fresh liquid egg yolk is stirred with 4 volumes of 95% aqueous ethanol (95% by volume of ethanol with 5% by volume of water) at a temperature of about 60°C for about 15 minutes. The slurry thus obtained is separated, for example by filtration, and the solid yolk residue is essentially yolk protein free from lipids. The residual aqueous ethanolic solution is subjected to low temperature crystallization, e.g. a tem-

perature of about 2°C to 5°C. The crystalline product which separates is essentially neutral triglycerides which is commonly known as neutral egg oil since it is crystalline at the temperature of 2°C to 5°C but liquefies at ambient temperature. Once the crystalline product has been removed, for example by filtration at that low temperature, the residual aqueous ethanolic solution may be dried, for example in a vacuum evaporator, to remove the aqueous ethanol and provide a final solid residue which is essentially egg lecithin.

Thus, according to a further feature of the invention, we provide a process for the separation of fresh liquid egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating about 100 parts by weight of fresh liquid egg yolk with about 400 volumes (1:4 weight to volume) of aqueous ethanol (5% by volume of water and 95% by volume of ethanol) at a temperature of about 60°C to provide a slurry thereof, separating said slurry, for example by filtration, to provide a solid fraction which is solid yolk protein and a liquid fraction which is an aqueous ethanolic solution, and thereafter subjecting said solution to low temperature crystallization to provide a crystalline neutral egg oil fraction and removing said crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

Best mode for carrying out the invention

According to yet an additional feature of the invention we provide a process for the separation of fresh liquid egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating about 100 parts by weight of fresh liquid egg yolk with about 400 volumes (1:4 weight to volume) of aqueous ethanol (5% by volume of water and 95% by volume of ethanol) at a temperature of about 60°C to provide a slurry thereof, separating said slurry, for example by

filtration, to provide a solid fraction which is solid yolk protein and a liquid fraction which is an aqueous ethanolic solution, and thereafter subjecting said solution to crystallization at a temperature of from about 2°C to about 5°C to provide a crystalline neutral egg oil fraction and removing said crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

By the use of the novel process of this invention it is possible to separate fresh liquid egg yolk from hens eggs into three products. The first product is yolk protein, the second product is neutral egg yolk oil so-called because it is an oil at ambient temperature and a crystalline product at a lower temperature of about 2°C to 5°C while the third product is egg lecithin. These products contain little or no undesirable byproducts and they are therefore very desirable products for use in the food and pharmaceutical industries.

The expression "aqueous ethanol" used herein is intended to mean ethanol containing from about 2% volume to volume of water (i.e. about 98% by volume of ethanol and about 2% by volume of water) to about 10% volume to volume of water (i.e. about 90% by volume of ethanol and about 10% by volume of water). A preferred aqueous ethanol is one containing from about 4% volume to volume to about 6% volume to volume of water, i.e. from about 96% by volume to about 94% by volume of ethanol, and, more especially, one containing about 5% volume to volume of water, i.e. 95% by volume of ethanol and 5% by volume of water. The volume (v) of aqueous ethanol used in the process in relation to the weight (w) of fresh liquid egg yolk used as starting material may vary from about 2 volumes, i.e. 2 v/w (volume to weight) to about 5 volumes, i.e. 5 v/w (volume to weight), and preferably from about 3 volumes to about 5 volumes (3 v/w to 5 v/w) and more particulary about 4 volumes, i.e. 4 v/w. In the latter case, it is preferred to use, for example, about 400 volumes of aqueous ethanol, desirably containing 95% by volume of ethanol and 5% by

volume of water, for about 100 parts by weight of fresh liquid egg yolk used as starting material thus giving a volume to weight ratio of about 4:1 v/w (volume to weight).

The expression elevated temperature is intended to mean a temperature of from about 45°C to about 75°C, preferably from about 55°C to about 65°C and more particularly a temperature of from about 58°C to about 62°C. A most preferred temperature is about 60°C.

The time taken for treatment of the fresh liquid egg yolk with aqueous ethanol to prepare a slurry thereof may vary from about 5 minutes to about 60 minutes but it has generally been found that the separation of the solid yolk protein fraction occurs fairly quickly. A treatment time of about 10 to 20 minutes, more particularly about 12 to 18 minutes, preferably about 15 minutes, provides a satisfactory separation.

As a preferred method of separation, it has been found that when starting with about 100 parts by weight of fresh liquid egg yolk and using about 400 volumes of about 95% aqueous ethanol (95% by volume of ethanol and 5% by volume of water), a preferred operating temperature is about 60°C and the time for treatment is generally about 15 minutes before the slurry thus obtained is ready for separation to separate the solid yolk residue from the aqueous ethanolic liquid.

Treatment of fresh liquid egg yolk with aqueous ethanol according to the process of this invention is effective in precipitating the solid yolk protein fraction while the residual aqueous ethanolic liquid contains essentially the neutral egg yolk oil and the lecithin. After removal of the solid yolk protein fraction, the residual aqueous ethanolic solution can be cooled to a sufficiently low temperature to permit the neutral egg yolk oil to crystallize out from the solution. A suitable low temperature below ambient temperature of from about 0°C to about 10°C, preferably from about 2°C to about 8°C, and

especially a temperature of from about 2°C to about 5°C. After the crystalline product has been removed, for example by filtration at the same low temperature, the residual aqueous ethanolic filtrate may be dried, for example by use of a rotary vacuum evaporator, to remove the water and ethanol under relatively mild conditions. The solid residue thus obtained is essentially lecithin which may be further purified, as required, by known means.

The crystalline product which is neutral egg oil in its crystalline state may be washed at low temperature with a small amount of cold aqueous ethanol to remove adhering traces of the filtrate from which it has been separated. The crystalline product may then be allowed to warm up to ambient temperature when it liquefies and the neutral egg yolk oil thus obtained is essentially a mixture of triglycerides. The oil may be further purified by a recrystallization procedure.

The originally isolated egg yolk protein may be retreated with aqueous ethanol to remove residual traces of undesired products and the slurry thus obtained may be filtered to obtain purified egg yolk protein.

It is to be understood that the expression "fresh liquid egg yolk" means unprocessed, unspoiled, natural liquid egg yolk, to be used as starting material, which has been obtained from hens eggs, irrespective of the age of said eggs.

The invention is illustrated by, but not limited by, the following Examples.

EXAMPLE 1

100 g Of fresh liquid egg yolk which contains approximately 50% of solid matter (i.e. about 50 g of solid matter and about 50 g of water), is diluted with 400 ml (1:4 weight to volume) of 95% aqueous ethanol (95% by volume of ethanol and 5% by volume of water, i.e. 95:5 v/v) in an extraction chamber and the mixture is stirred for 15 minutes at 60°C. There is thus obtained an aqueous ethanol egg yolk slurry. The slurry is filtered to provide a solid

residue which is essentially lipid-free egg yolk protein. The aqueous ethanolic filtrate contains egg yolk lipids (fat). The aqueous ethanolic filtrate is then cooled to a temperature of from about 2°C to about 5°C for a period of about 12 hours (roughly overnight). The neutral lipids (triglycerides) in the aqueous ethanolic filtrate crystallize out and are precipitated at the bottom. The bottom crystalline product (mainly triglycerides) is separated from the aqueous ethanol solution by filtering through a *Whatman #2 ashless filter paper. The crystalline product thus collected is transferred into a round bottomed flask with several rinsings of hot ethanol and is then dried (remove aqueous ethanol) by a rotary vacuum evaporator. The product is designated as "Neutral Egg Oil" (97.03% triglycerides).

The remaining aqueous ethanolic filtrate, after the removal of neutral egg oil (triglycerides), is dried in a rotary vacuum evaporator at 40°C. The solid fraction thus obtained is designated as "Egg Lecithin" (89.16% phospholipids).

The initially obtained solid yolk residue which is essentially lipid free egg yolk protein may be subjected to a further treatment with 100 ml of 95% aqueous ethanol in order to remove residual traces of lipids. The slurry thus produced is filtered and the solid residue of egg yolk protein thus obtained is free from lipids.

The neutral egg oil (97.03% triglycerides) contains only trace amounts (0.04% to 0.12%) of cholesterol. This cholesterol may be reduced or removed completely by subjecting the egg oil to a recrystallization procedure using aqueous ethanol or by subjecting the egg oil to a supercritical fluid extraction procedure.

EXAMPLE 2

In order to evaluate the variation in the rate of

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extraction over different time periods (15, 30 and 60 minutes) and at different temperatures (40°C and 60°C), the process described in Example 1 was further evaluated as follows:-

To 100 g of fresh liquid egg yolk (containing approximately 48.1% water, 35.0% lipids, 15.1% protein and 1.8% ash) were added 400 ml (1:4 weight to volume) of 95% aqueous ethanol (95% by volume of ethanol and 5% by volume of water). The slurry was homogenized in a blender and charged into the chamber of an extraction/filtration (EF) unit in the extraction mode. Extraction was continued by stirring for 15, 30 or 60 minutes. In filtration mode of the EF unit, the slurry was isothermally filtered at 40°C or 60°C with a gradually increasing pressure in order to prevent clogging of the filter. A triple layer of *FYNTEX 300 mesh (pore size 79 µm) nylon filter was found to be suitable for this purpose.

The aqueous ethanolic filtrate was collected and stored overnight (about 12 to 16 hours) at 2°C to 5°C. Two phases formed, one a crystalline precipitate at the bottom of the flask, which is essentially triglycerides, and an isotropic liquid phase at the top, which contains polar lipids, mainly lecithin. The two phases were separated by filtering using a *Whatman #2 ashless filter paper in a cold room temperature (2°C to 5°C). The crystalline phase containing neutral egg oil (triglycerides) and the liquid upper phase containing mainly polar lipids (lecithin) were each subjected separately to a drying process by evaporation at 40°C in a rotary vacuum evaporator.

It will be seen in the following tables that extraction at 60°C gives better results on the basis of the amount of products isolated. However, neither extraction rate nor purity of crude oil appeared to be influenced by the length of extraction time. A time of 15 minutes

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extraction under the above described conditions appeared to be satisfactory.

Effect of varying extraction time and temperature on fresh liquid egg yolk extraction with 95% aqueous ethanol.

		At 40°C		
		Time (min) 15	30	60
Lecithin	Yield (g)	11.16±1.10	10.92±0.44	12.15±1.37
	PL	10.17±0.97	9.88±0.48	10.85±1.44
	FC	0.71±0.07	0.77±0.03	0.75±0.11
	TG	0.25±0.10	0.23±0.02	0.40±0.31
Neutral Egg Oil	Yield (g)	18.20±1.10	17.57±0.91	17.31±1.08
	PL	1.02±0.49	0.92±0.15	0.66±0.18
	FC	0.06±0.02	0.06±0.01	0.04±0.04
	TG	17.13±0.90	16.58±0.77	16.60±1.28
Total Lipid Extracted (g)		29.35±1.19	28.49±0.82	29.45±2.44
Extractability (%)		83.90±3.41	81.44±2.34	84.18±6.99

		At 60°C		
		Time (min) 15	30	60
Lecithin	Yield (g)	11.04±0.36	11.76±0.23	11.39±0.63
	PL	9.79±0.24	10.42±0.29	10.16±0.52
	FC	0.88±0.09	0.82±0.25	0.71±0.19
	TG	0.33±0.05	0.36±0.08	0.44±0.20
Neutral Egg Oil	Yield (g)	22.68±0.32	22.72±1.11	21.77±0.41
	PL	1.34±0.10	1.19±0.08	0.90±0.18
	FC	0.11±0.01	0.12±0.09	0.04±0.03
	TG	21.22±0.43	21.40±1.18	20.85±0.31
Total Lipid Extraction (g)		33.72±0.68	34.48±1.19	33.16±0.43
Extractability (%)		96.36±1.93	98.54±3.39	94.77±1.24

PL = phospholipids (polar lipids containing lecithin).

FC = free cholesterol.

TG = triglycerides (neutral lipids).

EXAMPLE 3

In an attempt to try to determine the most suitable volume of aqueous ethanol to be used for the volume to weight (v/w) ratio, an experiment was carried out using 200, 300, 400 or 500 ml of 95% ethanol as follows:-

100 g Of fresh liquid egg yolk was extracted with 200, 300, 400 or 500 ml of 95% aqueous ethanol (95% by volume of ethanol and 5% by volume of water) at 60°C for 15 minutes. Extraction, filtration and cold temperature fractionation were performed as described in Example 2. As seen in the following table, a 4:1 volume to weight ratio gives 96.36% extractability, producing 11 g of crude lecithin and 22 g of neutral egg oil per 100 g of fresh liquid egg yolk, with a high purity. A ratio higher than 4:1 volume to weight (v/w) of 95% ethanol appears to increase the total extractability but the lecithin fraction becomes less pure mainly due to the shift of triglycerides to the lecithin fraction in the cold temperature fractionation step. Therefore, 400 ml per 100 g (4:1 v/w ratio) was adopted as the best procedure.

	95% aqueous ethanol volume (ml)			
	200	300	400	500
Crude (g) Lecithin	6.50±0.06	9.84±0.31	11.04±0.36	12.73±0.07
Crude (g) Oil	24.45±0.86	21.95±0.76	22.68±0.32	21.49±0.19
Total Lipids (g)	30.95±0.84	31.74±1.04	33.72±0.68	34.22±0.23
Extractability (%)	88.45±2.40	90.71±2.98	96.36±1.93	97.78±0.64

Various modifications and alterations of the process of this invention will become apparent to those skilled in the art without departing from the scope and spirit of this inventive process and it should be understood that this inventive process is not to be unduly limited by the illustrative embodiments and exemplification set forth herein.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the separation of fresh liquid egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating said fresh liquid egg yolk with aqueous ethanol at an elevated temperature to provide a slurry thereof, separating said slurry into a solid fraction and a liquid fraction wherein said solid fraction is solid yolk protein and said liquid fraction is an aqueous ethanolic solution, and thereafter subjecting said solution to low temperature crystallization to provide a crystalline neutral egg oil fraction and removing said crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

2. The process of claim 1 wherein said aqueous ethanol contains from about 2% volume to volume to about 10% volume to volume of water.

3. The process of claim 1 wherein said aqueous ethanol contains from about 4% volume to volume to about 6% volume to volume of water.

4. The process of claim 1 wherein said aqueous ethanol contains about 5% volume to volume of water.

5. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is from about 2:1 volume to weight to about 5:1 volume to weight.

6. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is from about 3:1 volume to weight to about 5:1 volume to weight.

7. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is about 4:1 volume to weight.

8. The process of claim 1 wherein there is used about 400 volumes of 95% aqueous ethanol for about 100 parts by weight of egg yolk, said aqueous ethanol containing 95% by volume of ethanol and 5% by volume of water.

9. The process of claim 1 wherein said elevated temperature is from about 45°C to about 75°C.

10. The process of claim 1 wherein said elevated temperature is from about 55°C to about 65°C.

11. The process of claim 1 wherein said elevated temperature is from about 58°C to about 62°C.

12. The process of claim 1 wherein said elevated temperature is about 60°C.

13. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is about 4:1 volume to weight and said elevated temperature is about 60°C.

14. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is about 4:1 volume to weight and said elevated temperature is about 60°C, said aqueous ethanol being about 95% aqueous ethanol.

15. The process of claim 1 wherein said treatment to provide said slurry takes from about 5 minutes to about 60 minutes.

16. The process of claim 1 wherein said treat-

ment to provide said slurry takes from about 10 minutes to about 20 minutes.

17. The process of claim 1 wherein said treatment to provide said slurry takes from about 12 minutes to about 18 minutes.

18. The process of claim 1 wherein said treatment to provide said slurry takes from about 15 minutes

19. The process of claim 1 wherein the ratio of said aqueous ethanol to egg yolk is about 4:1 volume to weight, said elevated temperature is about 60°C and said treatment to provide said slurry takes about 15 minutes, said aqueous ethanol being about 95% by volume of ethanol and 5% of volume of water.

20. The process of claim 4, 7 or 12 wherein said low temperature crystallization is carried out at a temperature of from about 0°C to about 10°C.

21. The process of claim 4, 7 or 12 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 8°C.

22. The process of claim 4, 7 or 12 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 5°C.

23. The process of claim 8, 14 or 19 wherein said low temperature crystallization is carried out at a temperature of from about 0°C to about 10°C.

24. The process of claim 8, 14 or 19 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 8°C.

25. The process of claim 8, 14 or 19 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 5°C.

26. A process for the separation of fresh liquid egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating about 100 parts by weight of fresh liquid egg yolk with about 400 volumes (4:1 v/w) of aqueous ethanol (5% by volume of water and 95% by volume of ethanol) at a temperature of about 60°C to provide a slurry thereof, separating said slurry into a solid fraction and a liquid fraction wherein said solid fraction is solid yolk protein and said liquid fraction is an aqueous ethanolic solution, and thereafter subjecting said solution to low temperature crystallization to provide a crystalline neutral egg oil fraction and removing said crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

27. The process of claim 26 wherein said low temperature crystallization is carried out at a temperature of from about 0°C to about 10°C.

28. The process of claim 26 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 8°C.

29. The process of claim 26 wherein said low temperature crystallization is carried out at a temperature of from about 2°C to about 5°C.

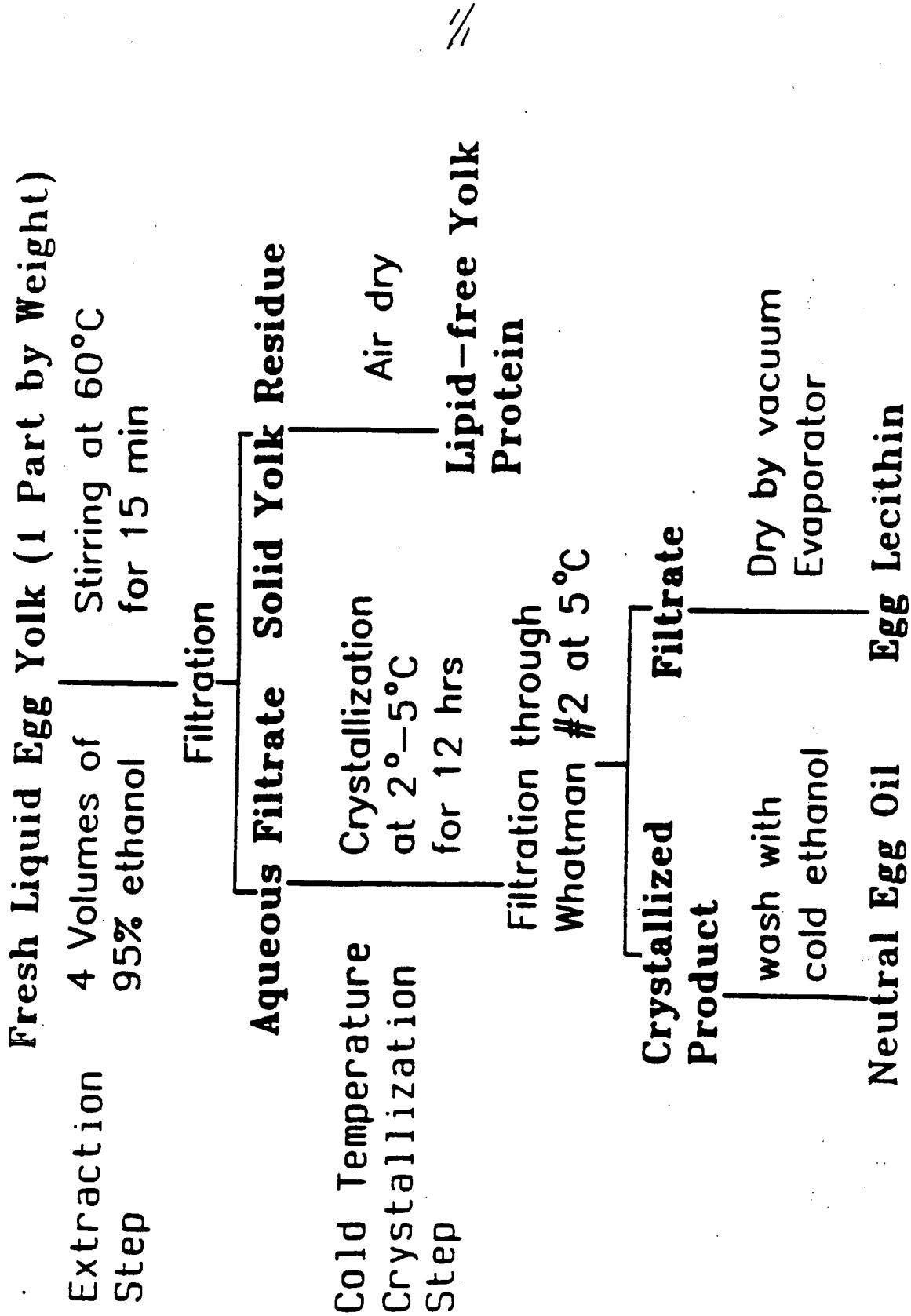
30. The process of claim 26 or 29 wherein said treating at a temperature of about 60°C is carried out for a period of about 15 minutes.

31. A process for the separation of fresh liquid

egg yolk into a yolk protein fraction, a neutral egg oil fraction and an egg lecithin fraction which comprises treating about 100 parts by weight of fresh liquid egg yolk with about 400 volumes (4:1 volume to weight) of aqueous ethanol (5% by volume of water and 95% by volume of ethanol) at a temperature of about 60°C to provide a slurry thereof, separating said slurry into a solid fraction and a liquid fraction wherein said solid fraction is solid yolk protein and said liquid fraction is an aqueous ethanolic solution, and thereafter subjecting said solution to crystallization at a temperature of from about 2°C to about 5°C to provide a crystalline neutral egg oil fraction and removing said crystalline fraction to provide a residual aqueous ethanolic solution containing egg lecithin.

32. The process of claim 31 wherein said treating at a temperature of about 60°C is carried out for a period of about 15 minutes.

33. The process of claim 1, 26 or 31 wherein the egg lecithin is isolated by removal of the aqueous ethanol.



INTERNATIONAL SEARCH REPORT

PCT/CA 90/00243

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 23 J 7/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 23 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4157404 (NOBUMITSU YANO) 5 June 1979 see experiment 1 --	1
Y	EP, A, 0259495 (SHIGEMATSU, Y) 16 March 1988 see page 4, lines 26-31 --	1
A	Patent Abstracts of Japan, volume 12, no. 37 (C-473)(2884), 4 February 1988, & JP, A, 62186744 (MORINAGA MILK INC. CO. LTD) 15 August 1987 see abstract --	1
A	Patent Abstracts of Japan, volume 12, no. 148 (C-493)(2995), 7 May 1988, & JP, A, 62263192 (NISSHIN OIL MILLS LTD) 16 November 1987 see abstract	1

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9000243 SA 38529

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 18/10/90
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